Prevention of intrauterine growth retardation by multiple micronutrient supplements during pregnancy in Burkina Faso

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Chapter 4  Effect of maternal multiple micronutrient supplements on cord blood hormones: a randomized controlled trial

also increased cortisol concentrations by 36% (P = 0.05). As in primiparae, this proportion was negligible. UNIMMAP supplementation had no significant effect on birth weight; however, UNIMMAP supplementation had a significant effect on cord blood in a subsample of 294 live single newborns. We performed mediation analysis with an Aroian test.

Results: UNIMMAP supplementation had no significant effect on insulin-like growth factor I (IGF-I), leptin, insulin, free thyroxine, or human chorionic gonadotropin concentrations in cord serum. However, UNIMMAP supplementation had a significant effect on insulin-like growth factor I (IGF-I), leptin, insulin, free thyroxine, and cortisol concentrations in cord serum in a subsample of 294 live single newborns. In these infants, IGF-I was shown to be particularly sensitive to maternal undernutrition in animal models (12, 13). The insulin-like growth factor (IGF) system is central in regulating fetal growth, particularly in early pregnancy and in the last trimester, with more maternal cortisol crossing to the fetus and a deletion of maternal malnutrition and/or anemia (21). Glucocorticoids, such as cortisol, have a beneficial effect on fetal organ maturation but may also have the potential to reduce fetal growth (13). In cases of maternal malnutrition, the activity of the placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) expression (24). Insulin also enhances cellular proliferation and differentiation, whereas IGF-II underexpression in the fetus may be influenced by insulin, insulin-like growth factor-I (IGF-I), which is the key mitogenic polypeptide stimulating fetal differentiation in the fetus (20) and might also be influenced by insulin, insulin-like growth factor-I (IGF-I), which is the key mitogenic polypeptide stimulating fetal differentiation in the fetus (20) and might also be influenced by the effect of UNIMMAP supplementation on maternal diet, or growth-retarded infants with normal growth. Offspring of primiparae could be related to constrained fetal growth. Confirmation of the effect of UNIMMAP supplementation on fetal growth. The specific hormonal pattern in fetal growth improves in pregnant women who take maternal multiple micronutrient supplements. In a double-blind, controlled trial carried out in Burkina Faso, we randomly assigned 1426 pregnant women to receive the Micronutriments et Sante´ de la Me`re et de l'Enfant Study (MISAME) Group.

INTRODUCTION

Intrauterine growth retardation (IUGR), defined as a birth weight less than the 10th percentile for gestational age and/or less than 2500 g); the remaining one-third of these LBW infants are born preterm, some of whom are also affected with IUGR (5). In developing countries, IUGR affects about two-thirds of the births of the general population, is an important predictor of mortality and morbidity in the neonatal period (1, 2) and during infancy (3) and may also have the potential to reduce fetal growth (13). In cases of maternal malnutrition, the activity of the placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) expression (24). Insulin also enhances cellular proliferation and differentiation, whereas IGF-II underexpression in the fetus may be influenced by insulin, insulin-like growth factor-I (IGF-I), which is the key mitogenic polypeptide stimulating fetal differentiation in the fetus (20) and might also be influenced by insulin, insulin-like growth factor-I (IGF-I), which is the key mitogenic polypeptide stimulating fetal differentiation in the fetus (20) and might also be influenced by the effect of UNIMMAP supplementation on maternal diet, or growth-retarded infants with normal growth. Offspring of primiparae could be related to constrained fetal growth. Confirmation of the effect of UNIMMAP supplementation on fetal growth. The specific hormonal pattern in fetal growth improves in pregnant women who take maternal multiple micronutrient supplements. In a double-blind, controlled trial carried out in Burkina Faso, we randomly assigned 1426 pregnant women to receive the Micronutriments et Sante´ de la Me`re et de l'Enfant Study (MISAME) Group.

Background: Fetal growth improves in pregnant women who take maternal multiple micronutrient supplements. In a double-blind, controlled trial carried out in Burkina Faso, we randomly assigned 1426 pregnant women to receive the Micronutriments et Sante´ de la Me`re et de l'Enfant Study (MISAME) Group.

Objective: Effect of maternal multiple micronutrient supplements on cord blood hormone concentrations. However, UNIMMAP supplementation had a significant effect on insulin-like growth factor I (IGF-I), leptin, insulin, free thyroxine, and cortisol concentrations in cord serum in a subsample of 294 live single newborns. In these infants, IGF-I was shown to be particularly sensitive to maternal undernutrition in animal models (12, 13). The insulin-like growth factor (IGF) system is central in regulating fetal growth, particularly in early pregnancy and in the last trimester, with more maternal cortisol crossing to the fetus and a deletion of maternal malnutrition and/or anemia (21). Glucocorticoids, such as cortisol, have a beneficial effect on fetal organ maturation but may also have the potential to reduce fetal growth (13). In cases of maternal malnutrition, the activity of the placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) expression (24). Insulin also enhances cellular proliferation and differentiation, whereas IGF-II underexpression in the fetus may be influenced by insulin, insulin-like growth factor-I (IGF-I), which is the key mitogenic polypeptide stimulating fetal differentiation in the fetus (20) and might also be influenced by insulin, insulin-like growth factor-I (IGF-I), which is the key mitogenic polypeptide stimulating fetal differentiation in the fetus (20) and might also be influenced by the effect of UNIMMAP supplementation on maternal diet, or growth-retarded infants with normal growth. Offspring of primiparae could be related to constrained fetal growth. Confirmation of the effect of UNIMMAP supplementation on fetal growth. The specific hormonal pattern in fetal growth improves in pregnant women who take maternal multiple micronutrient supplements. In a double-blind, controlled trial carried out in Burkina Faso, we randomly assigned 1426 pregnant women to receive the Micronutriments et Sante´ de la Me`re et de l'Enfant Study (MISAME) Group.

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Effect of maternal multiple micronutrient supplements on cord blood hormones: a randomized controlled trial1–4

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ABSTRACT

Background: Fetal growth improves in pregnant women who take daily maternal multiple micronutrients [United Nations International Multiple Micronutrient Preparation (UNIMMAP)] rather than iron and folic acid (IFA) alone.

Objective: Our objective was to test whether such an effect was mediated by changes in concentrations of cord hormones.

Design: In a double-blind, controlled trial carried out in Burkina Faso, we randomly assigned 1426 pregnant women to receive UNIMMAP or IFA supplements. We measured concentrations of insulin-like growth factor I (IGF-I), leptin, insulin, free thyroxine, and cortisol in cord serum in a subsample of 294 live single newborns. We performed mediation analysis with an Aroian test.

Results: UNIMMAP supplementation had no significant effect on cord hormone concentrations. However, UNIMMAP supplementation significantly affected concentrations of IGF-I (+30%; 95% CI: 8%, 52%; \( P = 0.009 \)) and leptin in male newborns. In these infants, 51.1% (\( P = 0.08 \)) of the effect of UNIMMAP supplementation on birth weight was mediated through IGF-I, whereas for female newborns, this proportion was negligible. UNIMMAP supplementation also increased cortisol concentrations by 36% (\( P = 0.009 \)) in cord blood in primiparae (\( P = 0.02 \)) and 25% higher (\( P = 0.05 \)) in cord cortisol in preterm, some of whom are also affected with IUGR (5). Maternal malnutrition is assumed to be a major determinant of IUGR (6–8), and it has been consistently observed in various settings that pregnant women who are provided with multiple micronutrient supplements deliver LBW infants less often (9–11). Although the physiologic mechanisms underlying such treatment effect are complex and not fully understood (Figure 1), an interaction with the hormonal regulation of fetal growth is particularly plausible (12, 13). The insulin-like growth factor (IGF) system is central in regulating fetal growth, particularly IGF-I, which is the key mitogenic polypeptide stimulating fetal cellular proliferation and differentiation, whereas IGF-II underpins embryonic growth (13, 14). IGF-I was shown to be particularly sensitive to maternal undernutrition in animal models (12, 14). In humans, a postnatal observational study (15) showed a positive correlation between birth size and micronutrient (ie, zinc and iron) and IGF-I concentrations in cord blood. Similar relations with maternal nutrition and fetal growth are suspected for leptin, a hormone secreted by adipocytes and by the placenta (16–19). Free thyroxine (T4) affects tissue accretion and differentiation in the fetus (20) and might also be influenced by maternal malnutrition and/or anemia (21). Glucocorticoids, such as cortisol, have a beneficial effect on fetal organ maturation but may also have the potential to reduce fetal growth (13). In cases of maternal malnutrition, the activity of the placental 11 \( \beta \)-hydroxysteroid dehydrogenase type 2 (11\( \beta \)-HSD2) is reduced with more maternal cortisol crossing to the fetus and a deleterious effect on fetal growth (22, 23). Chronic maternal deficiency of micronutrients such as zinc, copper, or vitamin E may alter placental 11\( \beta \)-HSD2 expression (24). Insulin also enhances tissue accretion via its anabolic effects on fetal metabolism (20).

INTRODUCTION

Intrauterine growth retardation (IUGR), defined as a birthweight-for-gestational-age below the 10th percentile of the reference population, is an important predictor of mortality and morbidity in the neonatal period (1, 2) and during infancy (3) and adulthood (4). In developing countries, IUGR affects about two-thirds of infants born with a low birth weight (LBW); birth weight <2500 g); the remaining one-third of these LBW infants are
and is sensitive to micronutrient provision. For instance, zinc is critical for insulin packaging and secretion (25), and vitamin E is an important up-regulator of insulin sensitivity (26). Cortisol (27, 28) and insulin (14) may also play a permissive role in fetal growth (ie, by acting on the IGF-I axis) (12).

Nutritional influences on hormonal regulation of fetal growth have been described mainly in animal studies with energy and protein restrictions (12, 14, 23–26) and in a few postnatal observational studies in human beings (15). Thus, the specific role played by maternal micronutrient supplements in humans remains grossly unknown to date. In addition, although 95% of IUGR cases are born in Asia and sub-Saharan Africa (29), information on the cord hormone concentrations in developing countries is scarce (15).

In an earlier study, we carried out a randomized controlled trial (30) in rural Burkina Faso to test the effect of daily supplementation with the United Nations International Multiple Micronutrient Preparation (UNIMMAP), designed by the United Nations Children’s Fund/World Health Organization/United Nations University (31), compared with daily supplementation with iron and folic acid (IFA). UNIMMAP supplementation resulted in increased birth weight and length (30). The treatment effect was more important in the upper part of the distribution of birth weight and length, which was consistent with results shown in a study in Nepal (32). There was also suggestive evidence of effect modification by parity, maternal body mass index, and type of malaria prevention (30).

Therefore, in the current study we tested the hypothesis that the effect of UNIMMAP supplementation observed on birth weight and length was mediated by hormonal changes in cord serum. In addition, we explored the relation between IGF-I, leptin, insulin, fT4, and cortisol in cord blood and anthropometric measures at birth.

SUBJECTS AND METHODS

Methods of the randomized controlled trial were described elsewhere (30). Briefly, between March 2004 and February 2006, 1426 pregnant women in the catchment area of 2 health centers, in Houndé district, Burkina Faso, were randomly assigned to receive either IFA (IFA group) or UNIMMAP (UNIMMAP group) daily. The intake of both supplements was directly observed by home visitors, and participants and assessors were blinded.

At enrollment, we measured maternal height, weight, arm circumference, and hemoglobin concentration in all participants. Gestational age was calculated on the basis of transabdominal ultrasound fetal biometry. When an ultrasound biometry was unavailable, the gestational age was computed on the basis of the last menstrual period. At delivery, newborn length, weight, occipito-frontal head circumference, and midupper arm circumference (MUAC) were measured twice. The mean of these 2 measurement sets taken within the first 24 h after birth were included in the analysis (33). Midwives collected cord blood immediately after delivery in the health center (ie, blood was not sampled if there was a home delivery). The midwives extracted 8 mL from the clamped cord with a syringe, transferred the blood in a polypropylene 10-mL dry tube without preservative
(Sarstedt 60.610.001; Sarstedt, Nümbrecht, Germany), and allowed it to clot at 4°C. The screwed tubes were transferred at 4°C twice a day to the central hospital (<8 km away) for centrifugation at 3000 rpm for 10 min and immediate serum freezing at −20°C. Hemolysis occurred in 12% of the cases, with an even occurrence between trial groups. The samples were kept frozen for a median duration of 20 mo. IGF-I can be fairly stably measured in serum with commonly used commercial assays (34) up to 9 y after the sampling (35). Cortisol, insulin, and thyroxine are stable in sera stored at −20°C (36). The long-term stability of leptin is poorly documented to date, although the stability is guaranteed in frozen dried blood spots (37).

Because IGF-I reportedly varies a lot in different populations, and there is currently no standard for sub-Saharan populations, the sample size was set to detect a theoretic difference of IGF-I concentration between UNIMMAP and IFA groups of 0.33 SD with 80% power and z = 5% (ie, 280 samples in total). On the basis of previous studies (38–41), a difference of 8 ng/mL between groups could be detected with such a sample size. However, insulin, cortisol, T4, and leptin could be measured only in half of the samples because of financial constraints. This resulted in an 80% power of detecting a difference of 0.66 SD between groups, which was still a reasonable effect size to observe. Eligible sera were restricted to single pregnancies to avoid extra variance because of discordant twin gestations (2% or 26/1286 of deliveries) (40). Samples to be analyzed were randomly selected from a coded list of available sera with a preset replacement strategy in case of hemolysis.

Sera were analyzed at the chemical laboratory of Liège University (Liège, Belgium) in February 2008. IGF-I was assayed by chemiluminescence immunoassay on the Liaison autoanalyzer (DiaSorin, Saluggia, Italy) with an analytic sensitivity of 3 ng/mL. Insulin, cortisol, and T4 were assayed by electrochemiluminescence immunoassay on the Modular Analytics E170 autoanalyzer (Roche Diagnostics, Mannheim, Germany) with an analytic sensitivity of 0.20 μU/mL, 0.18 μg/L, and 0.42 μg/dL, respectively. Leptin was assayed by sandwich enzyme immunoassay with the Quantikine human leptin kit (R&D Systems, Abingdon, United Kingdom) with an analytic sensitivity of 7.8 pg/mL. The intraassay CVs ranged from 0.6% to 3.1% but were slightly more elevated for IGF-I (3.1–4.5%). The interassay CVs all ranged from 1.2% to 3.3%.

This study was approved by the ethics committees of the Center Muraz (Bobo-Dioulasso, Burkina Faso) and the Institute of Tropical Medicine (Antwerp, Belgium).

Normality was assessed with a D’Agostino-Pearson’s test (SKTEST command in Stata 10.0; StataCorp, College Station, TX), and skewed variables were log transformed. Even after logtransformation, IGF-I was not totally normally distributed because 4% (n = 12 of 293) of the samples had a concentration under the limit of detection and thus displayed the same extreme value (3 ng/mL). Therefore, we created a sister variable where those extreme values were removed for sensitivity analysis in further models. Severe outliers were defined as hormonal values distant from the median by >3 interquartile ranges (42). There were 7 severe outliers (2 severe outliers for cortisol, insulin, and leptin and 1 severe outlier for IGF-I). They were primarily kept in the analysis to maximize data use. In a second stage, outliers were removed from the regression models for sensitivity analysis.

For each hormone, the analysis strategy followed 2 main axes, which resulted in the production of 2 main sets of models. The first axis (set I) focused on the relation between both maternal (IFA compared with UNIMMAP supplementation, primiparity, anthropometric measures at inclusion, and third pregnancy trimester during the lean season) and neonatal (sex and gestational age at delivery) characteristics and hormonal concentrations (dependent variables). Because hormones were log transformed, results were presented as the percentage change in hormones for one unitary change in the predictors. This corresponded to an elasticity in the form of (δlny)/(δx). For dichotomous independent variables, these elasticities can be interpreted as the percent change in the dependent variable associated with the presence of a positive indicator (eg, primiparous compared with not primiparous) (43). The consistency of results was checked by running logistic regression on dichotomized dependent variables (hormone concentrations in the upper tertile compared with the 2 lowest tertiles).

The second axis (set II) addressed the association between cord blood hormones (independent variables) and anthropometric variables at birth. Because LBW can result from shortness (symmetric growth retardation), thinness (asymmetric growth retardation), or both, which could result in different health outcomes (5), birth length and the Rohrer index (g/cm²) were also assessed. Head circumference and MUAC, an adequate predictor of whole-body fat mass (44), were also assessed. Because β coefficients of log transformed independent variables are sometimes difficult to interpret, we reported the effect on birth anthropometric measures of a 10% increase in the concentration of cord hormones, which was computed by β × 0.09531 [corresponding equations are shown in an article by Chiesa et al (45)].

In both sets, bivariate analysis was applied as a first step. This was followed by multivariate analysis in which all the variables with P < 0.10 in the bivariate analysis of set I were included. UNIMMAP supplementation was forced in every model of set I because of the primary hypothesis of the trial. In both sets, a backward stepwise procedure was applied with removal of variables if the likelihood ratio test yielded a P > 0.05 or a P > 0.10 for interaction terms (46, 47). Effect modification by birth weight (in tertiles) was also tested because the effect of UNIMMAP was shown to vary across the birth-weight distribution (30). The interaction between UNIMMAP and all other covariates of the models was tested systematically with a chunk test (48). In the case of a positive chunk test, specific significant interactions were looked at. As hormones do not work in isolation, in a last step we inserted all hormone variables in the models of set II and adjusted for the effect of sex, primiparity, gestational age at delivery, and maternal height to appraise the relative role of each hormone on birth anthropometric measures.

Multicollinearity was assessed by displaying the matrix-of-variance decomposition of the dependent variable (COLLDIAG command in Stata 10.0; StataCorp). Residuals compared with fitted-values plots were visually inspected to appraise the adequacy of the regression models.

Finally, we appraised by an Aroian test (49) the proportion of the effect of UNIMMAP supplementation on birth weight and length that was mediated through cord hormones, in particular IGF-I. Mediation through leptin was not tested because leptin both determines and results from fetal size, ie, fat mass. This was
an exploratory analysis because our study was not initially powered to test such mediation.

All analyses were adjusted for gestational age at delivery and for the type of malaria prevention (chloroquine compared with sulfadoxine-pyrimethamine) and catchment area to account for the study design (30). All analyses were done in Stata 10.0 (StataCorp).

RESULTS

Characteristics of mothers and offspring were similar to those previously described in the full data set (Table 1) [i.e., ~10% of all births were preterm, and 14.3% and 43.6% of infants were LBW and small-for-gestational-age (SGA), respectively (30)]. Only one important difference was noted in the primiparity rate, which was significantly lower in the control group in this sub-sample comparatively to the parent population. IGF-I and other hormones were measured in 294 and 139 cord blood samples, respectively. IGF-I, leptin, and insulin concentrations correlated positively, whereas cortisol was negatively correlated with IGF-I and insulin (Table 2). The concentration of fT4 was not correlated with any of the other hormones.

Set I

In bivariate analysis, UNIMMAP supplementation was not significantly associated with IGF-I, leptin, insulin, fT4, or cortisol concentrations. Four factors had an important influence on cord hormones. First, cord blood from primiparous women presented a different hormonal pattern with a significant reduction of IGF-I, insulin, and fT4, and the cortisol concentration increased by 25% (95% CI: 0%, 50%) compared with samples from multiparous women (Table 3). Second, short women (lowest height quartile) also presented a specific cord hormone pattern with pronounced decreased leptin and insulin concentrations and an elevated cortisol concentration. Third, male offspring had significant lower concentrations of IGF-I and leptin than female offspring. Finally, gestational age was associated with increased leptin and cortisol, and births that were premature resulted in 51% (95% CI: −7%, −95%) lower leptin concentrations.

In multivariate analysis, the effect of UNIMMAP supplementation on cord IGF-I was modified by sex and tertiles of birth weight (P for the triple interaction = 0.04). Because of the low power for subgroup analysis with our relatively limited sample, we presented the results graphically (Figure 2). Female newborns from both UNIMMAP and IFA groups exhibited similar IGF-I concentrations, whereas in male newborns, the difference of IGF-I concentrations between UNIMMAP and IFA receivers (overall difference: 30%; 95% CI: 8%, 52%; P = 0.009) increased with ascending birth weight (61%; 95% CI: 33%, 90%; P < 0.001) in the last tertile. Consistently, in logistic regression, the odds ratio of being in the upper tertile of IGF-I cord concentration when receiving UNIMAPP was 2.79 (95% CI: 1.30, 6.00; P = 0.008) in male newborns and 0.37 (95% CI: 0.18, 0.79; P = 0.011) in female newborns. The effect of UNIMMAP supplementation on leptin was also modified by sex (P for interaction = 0.068). Cord leptin increased by 37% (95% CI: 0%, 73%; P = 0.05) in males newborns from the UNIMMAP group, whereas cord leptin concentrations tended to decrease in female newborns (−29%; P = 0.09).

There was also a significant interaction between UNIMMAP intake and primiparity for cortisol concentration (P = 0.02). Cortisol concentrations were higher in cord blood from primiparous women who were given UNIMMAP (+36%; 95% CI: 9%, 63%; P = 0.009) but not in cord blood of their multiparous peers.

Set II

In bivariate analysis, IGF-I and leptin were positively associated with all birth anthropometric variables (Table 4). A 10% increase in the IGF-I concentration resulted in an increase of
TABLE 3
Regression analysis of the maternal and newborn characteristics associated with cord hormones (set I)

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>IGF-I (ng/mL) (n = 294)</th>
<th>Leptin (pg/mL) (n = 139)</th>
<th>Insulin (µU/mL) (n = 139)</th>
<th>fT4 (µg/dL) (n = 139)</th>
<th>Cortisol (µg/L) (n = 139)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Crude</td>
<td>Adjusted</td>
<td>Crude</td>
<td>Adjusted</td>
<td>Crude</td>
</tr>
<tr>
<td>UNIMMAP compared with IFA</td>
<td>4 (−11, 20)</td>
<td>M: 30 (8, 52)2,3</td>
<td>2 (−28, 24)</td>
<td>M: 37 (0, 73)6,7</td>
<td>−12 (−51, 27)</td>
</tr>
<tr>
<td>Mother’s height (cm)</td>
<td>−28 (−48, −7)3</td>
<td>−29 (−50, −8)3</td>
<td>−8.4 (−55, 38)</td>
<td>3 (1, 6)</td>
<td>6 (−24, −3)4</td>
</tr>
<tr>
<td>Mothers in the lowest height quartile</td>
<td>−12 (−31, 8)</td>
<td>−57 (−90, −24)2</td>
<td>−55 (−86, −22)2</td>
<td>1 (−7, 9)</td>
<td>20 (1, 38)5</td>
</tr>
<tr>
<td>Maternal BMI (kg/m²) at inclusion</td>
<td>2 (−2, 6)</td>
<td>5.7 (0, 12)5</td>
<td>0 (−9, 9)</td>
<td>0 (−1, 2)</td>
<td>−1 (−4, 2)</td>
</tr>
<tr>
<td>MUAC (mm) at inclusion</td>
<td>0 (−3, 4)</td>
<td>4 (−2, 10)</td>
<td>−3 (−12, 5)</td>
<td>1 (−1, 2)</td>
<td>−2 (−5, 1)</td>
</tr>
<tr>
<td>Third trimester in lean season</td>
<td>0 (−16, 16)</td>
<td>26 (0, 53)3</td>
<td>14 (−26, 55)</td>
<td>6 (−12, 0)3</td>
<td>6 (−13, 0)5</td>
</tr>
<tr>
<td>Maternal hemoglobin (g/dL) at inclusion</td>
<td>2 (−3, 6)</td>
<td>−8.8 (−17, 0)7</td>
<td>−10 (−22, 28)</td>
<td>1 (−2, 3)</td>
<td>1 (−4, 5)</td>
</tr>
<tr>
<td>Preterm birth</td>
<td>11 (−16, 39)</td>
<td>−51 (−95, −7)5</td>
<td>−45 (−89, −2)5</td>
<td>36 (−31, 103)</td>
<td>−9 (−20, 1)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>1 (−4, 2)</td>
<td>6.5 (2, 11)4</td>
<td>6.6 (2, 10)4</td>
<td>0 (−7, 6)</td>
<td>1 (0, 2)</td>
</tr>
<tr>
<td>Male newborn</td>
<td>−20 (−36, −4)3</td>
<td>−36 (−36, −10)3</td>
<td>−12 (−52, 27)</td>
<td>1 (−5, 7)</td>
<td>10 (−4, 24)</td>
</tr>
</tbody>
</table>

1 All values are mean percentages; 95% CIs in parentheses. Values are increases in hormone concentrations for a one-unit increase of the independent variable. IGF-I, insulin-like growth factor I; fT4, free thyroxine; UNIMMAP, United Nations International Multiple Micronutrient Preparation; IFA, iron and folic acid supplement; M, male newborns; F, female newborns; Mlt, multiparae; P, primiparae; MUAC, midupper arm circumference. All linear regressions were performed with log-transformed dependent variables. Crude analysis was conducted with one-by-one independent variables. Adjusted analysis included all variables with P < 0.10 at the crude (bivariate) analysis. Only variables for which the likelihood ratio test yielded P < 0.05 during the backward stepwise procedure were kept in the adjusted models. Gestational age at delivery, type of malaria prevention, and health center were variables forced in every multivariate model.

2 3 P = 0.043 for triple interaction with sex and tertile of birth weight.
3 4 P < 0.01.
4 5 P = 0.068 for interaction with sex.
5 6 P < 0.05.
6 7 P = 0.02 for interaction with primiparity.
7 8 P < 0.001.
0.36 mm (95% CI: 0.01, 0.73 mm) in birth length and in an increase of 10.5 mg/cm³ (95% CI: 5.9, 15.1 mg/cm³) increase in ponderal index. Moreover, IGF-I was 41.2% lower (95% CI: −26.0%, −56.4%; \( P < 0.00001 \); geometric mean = 23.8 ng/mL) and leptin was 27.3% lower (95% CI: −1.0%, −53.6%; \( P = 0.04 \)) in SGA newborns than in adequately grown infants (geometric means: 23.8 compared with 35.9 ng/mL for IGF-I and 2102.3 compared with 2763.8 pg/mL for leptin) (data not reported in Table 4). Insulin was positively associated with birth weight and the ponderal index, whereas fT4 was positively related to birth weight, length, MUAC, and head and chest circumferences. In multiple regression analysis, all of the observed associations of IGF-I and leptin with anthropometric variables remained valid, except for the association between leptin and head circumference. Insulin was not associated with birth weight after adjustment for other covariates. fT4 remained significantly associated with birth weight, length, and head and chest circumferences. When all hormone variables were inserted in the regression model, IGF-I and leptin remained independent predictors of birth weight (\( +14.1 \text{ g}; 95\% \text{ CI}: 2.4, 25.7 \text{ g}; P = 0.02 \) for a 10% increase in IGF-I; \( +12.9 \text{ g}; 95\% \text{ CI}: 4.4, 21.5 \text{ g}; P = 0.02 \) for a 10% increase in leptin). Leptin and fT4, but not IGF-I, were significant predictors of birth length (\( +0.72 \text{ mm}; 95\% \text{ CI}: 0.23, 1.20 \text{ mm}; P = 0.004 \) for a 10% increase in leptin; \( +2.18 \text{ mm}; 95\% \text{ CI}: 0.19, 4.17 \text{ mm}; P = 0.03 \) for a 10% increase in fT4). Only leptin remained associated with birth MUAC when the hormones were considered together (\( +0.26 \text{ mm}; 95\% \text{ CI}: 0.12, 4.9 \text{ mm}; P < 0.001 \)).

### Mediation

The Aroian test was restricted to cord samples issued from primiparous women (\( n = 244 \) of 294; 83%) because of the specific pattern of cord hormone concentrations detected in primiparous women in set I (Table 3). The test was also stratified by newborn sex given the modifying effect of sex detected in the set I analysis. Overall, 20.3% (\( P = 0.27 \)) of the effect of UNIMMAP supplementation on birth weight was mediated through IGF-I. However, the mediation effect was more important in male newborns than in female newborns. In male newborns, 51.1% (\( P = 0.08 \)) of the effect of UNIMMAP was mediated through IGF-I, whereas for female newborns, this proportion was negligible. A similar pattern was shown for birth length, with 32.5% (\( P = 0.16 \)) of mediation through IGF-I in male newborns and only 0.02% (\( P = 0.99 \)) for female newborns, although statistical significance was not reached in any of the groups. Because of the small sample size and the low power of mediation tests, the mediation of effect could not be computed concurrently by sex and birth-weight terciles. However, it was visually apparent that, similar to what was observed for IGF-I, UNIMMAP supplementation affected the distribution of terciles of birth weight differently by sex (Figure 3), with the proportion of newborns in the upper tercile of birth weight much more increased in male newborns (+10.8%; \( P = 0.02 \)) than in female newborns (+1.4%; \( P = 0.32 \)). Results were not significantly different after the removal of severe outliers from analysis or when using the IGF-I sister variable (results not shown).

### DISCUSSION

To our knowledge, this is the first study to assess the effect of prenatal micronutrients on hormonal concentrations in cord blood in humans. In addition, the associations between cord hormones and birth anthropometric measures were evaluated for the first time, to our knowledge, in an African population.

Overall, there was no evidence that UNIMMAP supplementation, compared with IFA supplementation, had an effect on cord hormone concentrations. The effect of multiple micronutrients may be mediated by other physiologic pathways than hormonal...
TABLE 4
Regression analysis of the effect of cord hormones on anthropometric indexes at birth (set II)

<table>
<thead>
<tr>
<th>Dependent variables</th>
<th>IGF-I (ng/mL)</th>
<th>Leptin (pg/mL)</th>
<th>Insulin (µU/mL)</th>
<th>FT4 (µg/dL)</th>
<th>Cortisol (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>
| Crude               | 17.0 (10.7, 23.3)
                   | 21.0 (13.2, 28.8)
                   | 5.86 (0.25, 11.5)
                   | 45.3 (9.57, 1.0)
                   | 1.85 (~12.1, 15.8)
| Adjusted            | 16.3 (10.2, 22.4)
                   | 15.9 (8.59, 23.3)
                   | 4.86 (~0.77, 10.5)
                   | 38.3 (2.09, 74.4)
                   | 2.31 (~11.6, 16.2)
| Birth length (mm)   |              |                |                |             |                |
| Crude               | 0.36 (0.01, 0.73)
                   | 0.73 (0.25, 1.21)
                   | 0.07 (~0.26, 0.40)
                   | 2.41 (0.34, 4.49)
                   | 0.77 (~0.13, 1.67)
| Adjusted            | 0.37 (0.01, 0.73)
                   | 0.51 (0.03, 0.98)
                   | 0.07 (~0.26, 0.40)
                   | 2.55 (0.43, 4.68)
                   | 0.55 (~0.32, 1.42)
| MUAC (mm)           | 0.36 (0.23, 0.48)
                   | 0.37 (0.22, 0.52)
                   | 0.06 (~0.04, 0.17)
                   | 0.34 (0.11, 1.46)
                   | 0.03 (~0.26, 0.33)
| Adjusted            | 0.33 (0.21, 0.46)
                   | 0.29 (0.14, 0.44)
                   | 0.06 (~0.04, 0.17)
                   | 0.64 (~0.04, 1.33)
                   | 0.008 (~0.27, 0.28)
| Ponderal index (mg/cm^3) | 10.5 (5.87, 15.1)
                   | 7.82 (1.84, 13.8)
                   | 4.75 (0.76, 8.74)
                   | 4.32 (~21.7, 30.4)
                   | ~9.73 (~20.8, 1.36)
| Adjusted            | 9.5 (4.8, 14.2)
                   | 6.71 (0.23, 13.2)
                   | 3.67 (~0.25, 7.60)
                   | ~4.87 (~30.5, 20.8)
                   | ~7.17 (~18.5, 4.19)
| Head circumference (mm) | 0.26 (0.06, 0.51)
                   | 0.34 (0.07, 0.60)
                   | 0.02 (~0.15, 0.21)
                   | 1.44 (0.30, 2.57)
                   | 0.30 (~0.19, 0.80)
| Adjusted            | 0.26 (0.04, 0.48)
                   | 0.19 (~0.07, 0.46)
                   | ~0.18 (~2.06, 1.71)
                   | 1.17 (0.026, 2.31)
                   | 0.30 (~0.18, 0.77)
| Chest circumference (mm) | 0.67 (0.38, 0.97)
                   | 0.72 (0.28, 1.14)
                   | 0.17 (~0.12, 0.46)
                   | 2.34 (0.51, 4.17)
                   | 0.16 (~0.64, 0.96)
| Adjusted            | 0.62 (0.32, 0.91)
                   | 0.46 (0.04, 0.89)
                   | 0.12 (~0.17, 0.41)
                   | 2.00 (0.15, 3.86)
                   | 0.04 (~0.73, 0.80)

\(^1\) All values are means; %5 CI's in parentheses. Values are the unit changes in dependent variables for a 1% increase in hormone variables. IGF-I, insulin-like growth factor I; FT4, free thyroxine; MUAC, midupper arm circumference. Crude analysis was performed with one-by-one independent variables. All linear regressions were performed with log-transformed independent variables. Adjusted analysis included all variables with \( P < 0.10 \) at the crude (bivariate) analysis. Only variables for which the likelihood ratio test yielded \( P < 0.05 \) during the backward stepwise procedure were kept in the adjusted models. IGF-I was adjusted for primiparity and newborn sex; leptin was adjusted for maternal height and infant sex; insulin and FT4 were adjusted for primiparity; and cortisol was adjusted for primiparity. United Nations International Multiple Micronutrient Preparation, and maternal height. Gestational age at delivery, type of malaria prevention, and health center were variables forced in every multivariate model.

\(^2\) \( P < 0.0001 \).

\(^3\) \( P < 0.05 \).

\(^4\) \( P < 0.001 \).

ones, such as increased nutrient availability to the placenta and fetus (directly or indirectly by enhancing appetite) (50), a lower incidence of maternal infections (51), less oxidative stress (52), the improvement of maternal hematologic variables (53, 54), or epigenetic gene regulation (55) (Figure 1). However, we cannot exclude that weak statistical power hindered the detection of an association between UNIMMAP supplementation and the concentration of the main hormones involved in fetal growth.

Another element that might have blurred the global picture was the effect modification of UNIMMAP supplementation by sex. UNIMMAP supplementation significantly affected IGF-I and leptin concentrations in male newborns only, and for IGF-I this effect increased with increasing birth weight. A differential role of micronutrient supplements in young male and female children was already suspected and could be explained by differences in sex steroids (56), although the link is still poorly understood. The higher IGF-I concentrations in male infants in the upper part of the birth-weight distribution in the UNIMMAP group could partly explain the observation in previous studies (30, 32) that the effect size of UNIMMAP supplementation on fetal growth also varied with percentiles of birth weight (ie, the effect was bigger in the upper part of the birth-weight distribution). Quite consistently, the proportion of the effect of UNIMMAP supplementation on birth weight mediated through IGF-I was significant only in male newborns. Because these elements and the congruent fact that UNIMMAP supplementation increased the proportion of newborns in the upper tertile of birth weight in male newborns only, the overall effect of UNIMMAP supplementation on birth weight was not significantly modified by sex.

The effect of UNIMMAP supplementation was also modified by primiparity, with the concentration of cord cortisol being significantly increased in primiparous women receiving UNIMMAP instead of IFA. This was an unexpected finding because micronutrient supplementation is generally assumed to enhance the 11β-HSD2 expression in deficient women (24) and thus to decrease the cortisol exposure to the fetus, as this tended to be the case in multiparous women in our study. However, this function of micronutrients is still debated, and zinc, for instance, could be a blocker rather than an enhancer of the 11β-HSD2 (57). Why the effect of UNIMMAP supplementation on 11β-HSD2 expression might differ in primiparous and multiparous women is unknown. However, it is noteworthy that UNIMMAP also had a differential effect on fetal growth by parity; ie, UNIMMAP supplementation failed to improve fetal growth in primiparous women in a number of nutritional intervention studies (30, 58, 59), including the one we led in the same study population (30). Because cortisol concentration was negatively related to IGF-I concentration, the higher cortisol concentrations observed in primiparous women might have exerted a control on fetal growth via a modulation of IGF-I secretion (27). We also reported that the perinatal mortality was marginally significantly increased by UNIMMAP supplementation in primiparous women (30), which is a striking observation even if the link between these 2 observations is unclear.
Besides investigating the effect of UNIMMAP supplementation on cord hormone concentrations, our study also permitted study of the relations of such hormones with maternal and newborn indicators in an African population. First, there was a specific hormonal pattern in the cord blood from primiparous women. IGF-I, insulin, and FT4 were lower, and cortisol was higher, than in samples from multiparous women. Such differences could explain, at least partly, differences in the clinical status of primiparous offspring who are generally smaller and suffer a higher perinatal death rate (60, 61). Cord cortisol is a stress marker and has been shown to be higher in primiparous women (62). Second, concentrations of IGF-I and leptin were lower in male newborns, which is an observation that agrees with a well-known sexual dimorphism (63, 64). Such a gradient could be explained by the transient perinatal elevation in sex hormones (65), whereas the higher leptin concentrations in female newborns could also be explained by a different fat distribution (63). Last, we showed that cord IGF-I and leptin were independent predictors of birth weight. This was consistent with previous reports (18, 66) and suggests that different hormonal mechanisms affect birth weight. However, for leptin, a reversed causality is possible as hormonal concentrations were higher because of a higher fat mass. In our study, cord leptin was positively associated with MUAC at birth. Because the MUAC can be regarded as a proxy for a newborn’s fat mass (44), this result fits with the acknowledged link between fetal fat mass and cord leptin concentrations (67). However, the placenta is also a source of leptin (68), and may confound that relation. Cord leptin was also associated with birth length in our study, which could be explained by the reported role of leptin on bone mass formation (19, 69). In contrast IGF-I was no longer related to birth length in the multivariate analysis. This was an unexpected finding given that IGF-I is usually considered the main driver of fetal growth (70). However, in most previous studies, fetal growth has only been defined in terms of birth weight and not birth length. Lo et al (71) showed results similar to ours, whereas Vatten et al (18) reported that leptin and IGF-I were independent predictors of birth length, although in the study of Vatten et al (18), only term infants were included, and no tables of evidence were provided.

Insulin was not associated with fetal size in multivariate analysis, despite its positive correlation with IGF-I and leptin concentrations. This is agreement with what is known about its permissive role in fetal growth (ie, insulin increases glucose uptake and the cellular availability of glucose) (12, 17) but has no direct role on growth. Insulin also drives the deposition of fat in the fetus in late gestation, which possibly coregulates leptin (14). FT4 had a significant association with birth length that was independent of leptin. The link between cord FT4 and birth weight was already observed (72), but to our knowledge, this is the first time that the specific association with birth length is acknowledged.

Our study presented some limitations. The sample size was relatively limited, and thus mediation analysis was exploratory. Also, the comparator was IFA, which directly exerts an effect on maternal hemoglobin (54) and fetal growth (73). This could have resulted in smaller differences than expected between groups.

In conclusion, this study identified sex-specific effects of maternal multiple micronutrient supplementation on IGF-I and leptin concentrations in cord blood. Moreover, cord hormones from primiparous women had a specific pattern that could partly explain the lower birth size of their offspring. In addition, IGF-I and leptin concentrations in cord blood were independently associated with birth size. This study paved the way for a better understanding of mechanisms linking maternal nutrition and fetal growth, but given its explorative nature, replication in a hypothesis-confirming study is warranted.

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